McNeil et al. *New Zealand Plant Protection 76* (2023) 29-34
https://doi.org/10.30843/nzpp.2023.76.11773
Published: 25/09/2023

RESEARCH NOTE

Timing is everything: Improving predictions of winter New Zealand grass grub densities and associated damage from summer and autumn larval counts

Mark R. McNeill*, Russell G. Croy, Chikako van Koten and Shengjing Shi

AgResearch Ltd., Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand

**Corresponding author:* mark.mcneill@agreseach.co.nz

(Original submission received 27 April 2023; accepted in revised form 11 September 2023)

Summary *Costelytra giveni* is a serious pasture pest in New Zealand and accurate estimates of population densities are important to inform control measures. This species generally has a one-year life cycle so populations should either remain stable after eggs have hatched or decline due to larval mortality. Larval counts were obtained using a simple, standard and widely used sampling method from a series of soil cores collected from ryegrass research plots in Canterbury, New Zealand between 27 January and 16 June 2021 and a significant increase in population was recorded. Measurements on 27 January, 19 March and 5 May, represented only c. 8%, 25% and 63% of the mean densities measured on 16 June, respectively. The apparent increase in larvae is attributed to failure to find small 1st and 2nd instar individuals within the soil samples. Larvae increased in size as they transitioned from 1st to 3rd instar and later instar specimens were more easily discovered. An equation to describe the observed results provided date-related correction factors to allow a more realistic prediction of *C. giveni* larval densities in the winter following empirical larval counts. Larval counts measured on 27 January, 19 March, and 5 May, would need to be multiplied by 13, 4 and 1.6, respectively, to accurately estimate the larval density found on 16 June. This study showed that summer-autumn sampling using the current method can significantly underestimate winter *C. giveni* larval densities, potentially leading to unanticipated pasture production losses. Similar results were also found in 2022. While the equation provides a guide to population estimates, the caveat is that region and environment will influence population trends in any particular year.

Keywords *Costelytra giveni*, modelling, biopesticides, pasture pest, pest prediction

INTRODUCTION

Grass grub (*Costelytra giveni*, Coca-Abia & Romero-Samper), previously *C. zealandica* (Coca-Abia & Romero-Samper, 2016), is New Zealand's number-one pastoral pest, impacting on pasture productivity, persistence, and quality (Ferguson et al. 2019), with estimated financial losses in the order of NZ$156–425M pa. (McNeill et al. 2021). Larvae of this New Zealand endemic scarab feed on the roots of grasses and clovers (Dumbleton 1942; Radcliffe 1970; Ferguson et al. 2019). The larvae are C-shaped, generally white in colour but often translucent allowing soil to be seen in the hindgut, with a light tan head and a horseshoe-shaped cluster of anal bristles. *Costelytra giveni* generally has a one-year life cycle, with three larval instars. Newly hatched larvae are c. 5 mm long, weighing 2-3 mg and, when fully grown, third instar larvae are 20-25 mm long and weigh 150-250 mg (Fenemore 1984). Adult females lay the bulk of their eggs near where they emerge from the soil following eclosion, resulting in aggregated larval populations and associated patches of damaged pasture. Larval populations generally build up to damaging levels within 2-5 years after pastures are sown following cultivation (East and Kain 1982; Jackson et al. 1989; Ferguson et al. 2019) although occasionally damage can also occur to older pastures. In Canterbury, larval damage to pasture is most obvious in winter (June-July) but estimating larval densities to determine potential damage can be undertaken in March (autumn) to predict peak densities in April-May (Ferguson et al. 2019), allowing planning and implementation of mitigation measures before significant pasture damage occurs. Threshold densities at which damage is noticeable have been determined to be between c. 150 to 200 larvae m^{-2} depending on farm type and soil moisture, with control options ranging from destocking through to insecticide application (Ferguson et al. 2019).

Sampling larval populations is usually done by digging holes in the pasture and sorting the resultant soil by hand to extract the larvae. This method is widely used because it is simple to undertake but manual extraction of early-instar larvae may be underestimated because of their small size. Underestimation of larval populations can have serious consequences for pasture productivity but no studies have examined the limitations of the manual extraction method for estimating grass grub populations.

MATERIALS AND METHODS

Site details

The trial site was located on the AgResearch, Lincoln research farm (S43.632°, E172.469°) near Lincoln, Canterbury. The soil type was a Wakanui imperfectly drained silt loam (mottled Immature Pallic Soil) with two thirds being Wakanui sibling 3 and the bottom third Wakanui sibling 6 (Manaaki Whenua - Landcare Research 2019). A seed bed was prepared by applying glyphosate (1,470 g/ha) in July 2020 to the existing pasture then, in August, ploughing to a depth of 15-20 cm, harrowing and rolling. The top 5-10 cm was cultivated with a power harrow in early September to create a firm fine seedbed prior to sowing. This seed bed preparation would have eliminated any larvae that were present in the previous pasture (Stewart 1986).

Six monoculture pasture treatments were sown over 14-23 September 2020 in a randomised block design with three replicates. Plots measured 50 m x 32.5 m.

Establishment of plots

The seeding rate was 25 kg/ha sown with a Duncan Renovator drill at 15 cm row spacings. Irrigation was applied throughout October 2020 to ensure good pasture establishment and between 1 January and 1 May 2021 when three applications of 15-20 mm per pass were made. On 6 November 2020, all pastures were sprayed with a mixture of Tropotox, Versatill and Preside herbicides (1,125 g/ha MCPB, 75 g/ha MCPA, 150 g/ha clopyralid, 52 g/ha flumetsulam) for top control of broadleaf weeds. Nitrogen fertiliser in the form of urea or ammonium sulphate was applied at 40 kg N/ha on 6 January 2021, 30 March, 25 June, 26 August, and 6 October 2021.

The experimental plots were grazed periodically by sheep in February-March (42 days), May-June (30 days), Sept-Oct (28 days) and Nov-Dec 2021 (40 days), as described by Croy et al. (2022).

Pasture treatments comprised of a combination of three perennial ryegrass (*L. perenne* L.) cultivars and three *Epichloë* endophyte strains (*E. festucae* var. *lolii* (Latch, M.J.Chr. & Samuels) C.W.Bacon & Schardl and *Epichloë* species (LpTG-3), and an endophyte-free perennial ryegrass treatment. However, the results presented here are across all treatments.

The proportion of tillers infected with endophyte was assessed in January and November 2021, with 50 ryegrass tillers randomly selected from within each plot and endophyte status of each tiller assessed using an enzyme-linked immunosorbent assay (ELISA) (Simpson et al. 2012). Tiller endophyte infection levels were uniformly high (80 – 98%) across all ryegrass-endophyte combinations. (R. Croy, unpublished data).

Larval Sampling

2021

Sampling for *C. giveni* larvae occurred on 27 January (summer), 19 March (autumn), 5 May (autumn), 16 June (winter), 4 November (spring) and 21 December (summer) 2021. Ten geolocated sample points were randomly allocated to each plot using ARCGIS Pro software. Geolocation allowed for repeated soil sampling from within a 0.4 m dia. area around each point. Samples were collected using a 10 cm dia. handheld corer to a depth of 10-12 cm, with one core per point taken per sampling occasion. The vegetation on each core was removed to soil level with hand shears and the core placed individually in plastic bags. The cores were then hand sorted in the laboratory by several operators with previous experience extracting *C. giveni* larvae. Soil and root mass was manually crumbled and larvae found, removed and counted. By November 2021, larvae had developed into pupae and adults, and those data are not considered here.

2022

A nearby paddock on the AgResearch farm was sampled separately the following year and counts undertaken by just one experienced operator. The site was sown with perennial ryegrass (cv. One50) with AR1 endophyte in April 2020. Red clover breeding lines along with commercial cultivars Broadway, Colenso, Relish and Sensation were transplanted into the monoculture in September 2020. Soil core samples were collected on 22 February, 19 April, 26 May and 27 June 2022.

Statistical analysis

Larval counts of the 10 soil cores taken from each plot were summed to calculate the total count for the plot, then converted into densities (n/m^2). Larval densities were $\log_e(x+1)$-transformed to stabilise variation, and to approximate their distribution closer to a normal distribution. The addition of 1 to the densities was required to include observed zero densities in the analysis. The transformed densities were analysed using a linear mixed model (LMM) with statistical software SAS (version 9.4). The correlation among repeatedly measured densities were treated as a random effect. A single common trend across all six treatments was estimated.

RESULTS AND DISCUSSION

Costelytra giveni most often has a one-year life cycle with occasional two-year life cycles occurring under cool or very dry conditions (Fenemore 1984) or at southern latitudes (Stewart & Stockdill 1972). Egg laying and egg hatch are both closely synchronised and multiple generations within a year have not been observed. For one-year lifecycle grass grub in Canterbury, 1st instar larvae are present from early December to early February, 2nd instar larvae from mid-January to early April and 3rd instar larvae from early March

until early October. The 1st instar larvae feed in the immediate vicinity of plant roots and 2nd instar larvae in the top 5 cm of soil before moving to 7.5 cm as they become 3rd instar larvae (Pottinger 1968). The entire larval phase, therefore, occurs within the soil zone covered by the sampling technique and larval densities are expected to remain constant or decline slightly over time due to some natural mortality.

Unexpectedly, density estimates of *C. giveni* larval populations significantly increased across all treatments from January to June 2021 (P=0.047 – P<0.001), Table 1. Peak mean (± SEM) densities were recorded in the June sampling and ranged from 34.0 ± 8.47 to 314.3 ± 37.71 larvae m^{-2}, across treatments. Reduced herbage production was found in some plots supporting high larval populations, with lower herbage production requiring lower stocking rates during grazing (R. Croy, pers. comm.). Differences among treatments were found but cannot be reported at this time.

The larval density data were modelled across all grass-endophyte treatments. The overall trend to describe the mean change in estimated *C. giveni* larval density was given as: Log_e(Larval density+1) = 1.819 + 0.020*Days from 1 January (Figure 1). Estimates based on empirical density data as well as intervening modelled weekly density estimates are shown in Table 1. The modelled trend line indicates that mean larval populations measured on 27 January and 19 March, represented only c. 8 and 25% of the densities measured on 16 June, respectively (Figure 1). Furthermore, the average population measured on 5 May was only 63% of the population measured on 16 June. To estimate the larval density empirically obtained on 16 June, the mean counts measured on 27 January, 19 March, and 5 May, would need to be multiplied by 13, 4 and 1.6 x, respectively. The 16 June empirical data are likely to be the most accurate as the larvae were an average of third instar. Larvae are 20-25 mm long at this time but even this data point could still be an underestimation of the actual population density. If so, then the earlier data may represent even greater underestimation.

As the population was from a one-year life cycle, the apparent increase in density (Figure 1) over time is therefore a false result. The most obvious and only conclusion that can be made is that smaller larvae were not found by the standard hand-sorting extraction method used. This underestimation occurred despite laboratory conditions providing a more comfortable working environment for larval sorting and counting which is often not the case while field sampling. It is logical to assume that larvae were more easily found in cores sampled later as they transitioned from 1st to 2nd and then 3rd instar, and increased in body size. Another possible issue is operator variation as several staff were involved in the processing of cores but all had prior experience with extracting *C. giveni* larvae.

The implication from these data is that, without correcting for missed larvae, early assessments in February – April significantly underestimate true population density and, therefore, underestimates the potential severity of grass grub damage predicted to occur in winter. These results could have simply been attributed to site and season so a nearby paddock on the AgResearch farm was sampled separately the following year and counts undertaken by just one operator. Mean (± SEM) estimated density was 18.2 ± 18.88 larvae m^{-2} on 22 February and 136.4 ± 56.36 larvae m^{-2} on 19 April 2022. This difference represents an apparent 7.5 x increase in density over a 54 day period and, while greater than changes observed in 2021, the SEM of the 19 April data point still overlapped with the confidence interval (CI) limit of the estimated trend. Interestingly, subsequent sampling on 26 May found that the population had collapsed to 16.0 ± 11.20 m^{-2}, which was confirmed when sampling on 27 June 2022 found that the mean density was only 7.1 ± 7.72 larvae m^{-2}. The population decline is attributed to an epizootic event caused by one of the entomopathogens associated with *C. giveni* (Glare et al. 1993). This epizootic event illustrates that other factors may also need to be considered when generating an equation to accurately model grass grub

Table 1 Measured and estimated *C. giveni* larval densities (m^{-2}) by week per month, with lower and upper confidence interval measured on a Canterbury sheep research farm from 27 January (day 27) to 16 June (167) 2021. Actual sample dates indicated in bold italics.

Date	Days	Density	Confidence Interval	
			Lower	Upper
27 January	***27***	***9.6***	***5.5***	***16.1***
1 February	31	10.5	6.1	17.4
7	38	12.2	7.3	19.9
14	45	14.2	8.7	22.7
21	52	16.4	10.3	25.9
27	58	18.7	11.8	29.1
1 March	60	19.5	12.4	30.3
7	66	22.1	14.2	34.0
14	73	25.5	16.6	38.9
19	***78***	***28.3***	***18.6***	***43.0***
21	80	29.5	19.4	44.7
27	86	33.4	22.1	50.3
1 April	91	37.1	24.6	55.6
7	97	41.9	27.9	62.8
14	104	48.4	32.2	72.4
21	111	55.8	37.1	83.6
27	117	63.0	41.8	94.7
1 May	121	68.3	45.3	102.9
5	***125***	***74.1***	***49.0***	***111.9***
7	127	77.2	50.9	116.8
14	134	88.9	58.2	135.5
21	141	102.4	66.5	157.5
27	147	115.6	74.5	179.3
1 June	152	127.9	81.7	199.9
7	158	144.3	91.3	227.9
14	165	166.2	103.7	265.9
16	***167***	***173.0***	***107.5***	***277.9***

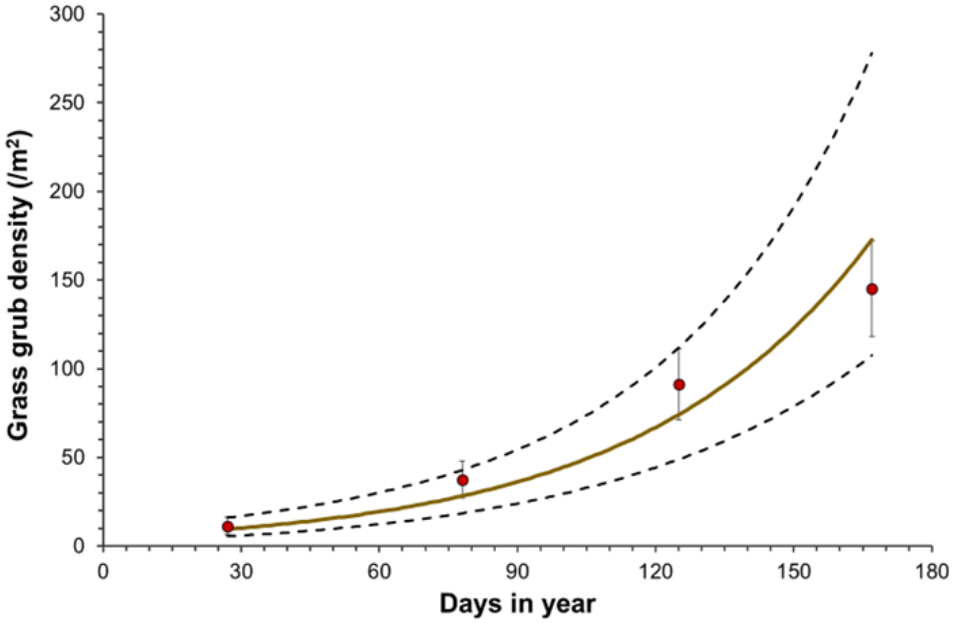

Figure 1 Trend line (solid line) with the 95% confidence boundaries (dashed lines), describing the estimated mean *C. giveni* larval densities (red circles ± SEM) across all grass-endophyte treatments measured on a Canterbury sheep research farm on 27 January (day 27), 19 March (78), 5 May (125) and 16 June (167) 2021. The trend line was generated using the equation Log_e (Larval density+1) = 1.819 + 0.020*Days from 1 January 2021. To maximise the certainty of prediction, the estimated upper 95% confidence density value should be used.

numbers and predicted damage. Such factors will include entomopathogen levels (Glare et al. 1993, Hurst et al. 2021), soil moisture level (Kain 1975, East and Willoughby 1980), sward composition (Kain and Atkinson 1977), larval mortality (Kain 1975) and pasture management (East and Pottinger 1983).

All these influencing factors reduce population density and population decline over time which has been observed by van Toor and Dodds (1994) in Southland using similar methodology. Their data emphasise that yearly changes in larval densities are a function of pasture age and the presence of entomopathogens in the soil. Understanding the interactions between these factors is important to accurately predicting population trends and impacts, but obviously requires further research.

One-year pasture has traditionally been considered too young for grass grub densities to reach levels of 150-200 larvae m^{-2} where pasture damage is likely to occur (Ferguson et al. 2019). However, estimated population densities found in the first year following pasture establishment in the current study were high enough to be potentially damaging to the pasture. Therefore, the results from the current study indicate that monitoring pastures for the presence of damaging populations of *C. giveni* may need to occur earlier in the life of pasture than previously suggested (e.g., Jackson et al. 1989; Ferguson et al. 2019).

The pseudo-increase in *C. giveni* larval densities observed over time in the current study may also hold true if the same or similar sampling method is used for other scarab species such as manuka beetle (*Pyronota festiva* F. and *P. setosa* Given), Tasmanian grass grub (*Acrossidius tasmaniae* Hope) and African black beetle (*Heteronychus arator* F.), as well as other orders of pasture insects e.g., porina (*Wiseana* spp).

CONCLUSIONS

The standard, simple and widely used method for measuring grass grub larval populations in the field used here allows consultants and farmers to assess paddocks easily. However, this study showed that sample timing can have a significant effect on estimates of *C. giveni* larval densities using this method. Without applying a correction factor to compensate for larvae that are missed during counting, data from sampling in summer (February) through to autumn (May) will underestimate grass grub population in the winter (June onwards), potentially leading to unanticipated pasture production losses. Applying the equation Log_e(Larval density+1) = 1.819 + 0.020*Days from 1 January to data collected in summer and early autumn provides a simple method to estimate *C. giveni* larval densities in late autumn – winter. In addition, it is also important to use the estimated upper 95% confidence density value rather than the mean against the control threshold, to maximise the certainty of prediction. Access to more accurate estimates will allow farmers time to plan management strategies to mitigate the impacts of high larval densities. However, the caveat to applying such an equation is that region, environment, pasture age and the incidence of entomopathogens in the soil will influence population trends in any particular year.

ACKNOWLEDGEMENTS

The authors thank Lee Sutherland, Sandra Young, Emily Gerard, Mary Ann Tuboltsev, Jill Gibson and Kritarth Seth of AgResearch for their assistance in the collection and processing of samples. Colin Ferguson and David Hume of AgResearch provided valuable comments on an earlier draft of the manuscript. We appreciate permissions granted by

Grasslanz Technology Ltd and PGG Wrightson Seeds Ltd for access to the field trial site for sampling. The original field research was supported through the AgResearch Strategic Science Investment Fund via the New Zealand Ministry of Business Innovation and Employment (Contract No. A25941 "Microbiomes from soil to plate").

REFERENCES

Croy RG, Sutherland BL, Hume DE, Mace WJ, C van Koten C, Finch SC 2022. Animal safety of a tall fescue endophyte (*Epichloë* sp.) in a perennial ryegrass (*Lolium perenne*) host. New Zealand Veterinary Journal 70(3): 165-176. https://doi.org/10.1080/00480169.2021.2011795

Coca-Abia MM, Romero-Samper J 2016. Establishment of the identity of *Costelytra zealandica* (White 1846) a species commonly known as the New Zealand grass grub. New Zealand Entomologist 39(2): 129-146. https://doi.org/10.1080/00779962.2016.1230254

Dumbleton LJ 1942. The grass grub (*Odontria zealandica* White): A review of the problem in New Zealand. The New Zealand Journal of Science and Technology 23(6A): 305-321.

East R, Kain WM 1982. Prediction of grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae) populations. New Zealand Entomologist. 7(3): 222-227. https://doi.org/10.1080/00779962.1982.9722385

East R, Pottinger R 1983. Use of grazing animals to control insect pests of pasture. New Zealand Entomologist 7(4): 352-359. https://doi.org/10.1080/00779962.1983.9722423

East R. Willoughby BE 1980. Effects of pasture defoliation in summer on grass grub (*Costelytra zealandica*) populations. New Zealand Journal of Agricultural Research 23: 547-562. https://doi.org/10.1080/00288233.1980.10417882

Fenemore PG 1984. Grass grub, *Costelytra zealandica* (White), life cycle. DSIR Information Series No. 105/4; 1st revision. J.A. Wightman ISSN 0077-9636, 4 pp. http://digitallibrary.landcareresearch.co.nz/digital/collection/p20022coll21/id/52

Ferguson CM, Barratt BIP, Bell N, Goldson SL, Hardwick S, Jackson M, Jackson TA, Phillips CB, Popay AJ, Rennie G, Sinclair S, Townsend R, Wilson M 2019. Quantifying the economic cost of invertebrate pests to New Zealand's pastoral industry. New Zealand Journal of Agricultural Research 62(3): 255-315. https://doi.org/10.1080/00288233.2018.1478860

Glare TR, O'Callaghan M, Wigley PJ 1993. Checklist of naturally occurring entomopathogenic microbes and nematodes in New Zealand. New Zealand Journal of Zoology 20(2): 95-120. https://doi.org/10.1080/03014223.1993.10422867

Hurst MR, Beattie A, Laugraud A, Townsend R, Sitter L, van Koten C, Harper L 2021. Identification of diverse toxin complex clusters and an eCIS variant in *Serratia proteamaculans* pathovars of the New Zealand grass grub (*Costelytra giveni*) and Manuka beetle (*Pyronota* spp.) larvae. Microbiology Spectrum 9(2): e01123-21. https://doi.org/10.1128/Spectrum.01123-21

Jackson TA, Barlow ND, Pearson JP, French RA 1989. Effect of paddock rotation practices on grass grub (*Costelytra zealandica*) damage in Canterbury. Proceedings 5th Australasian Grassland Invertebrate Ecological Conference 1988: 146-152.

Kain WM 1975. Population dynamics and pest assessment studies of grass grub (*Costelytra zealandica* (White), Melolonthinae) in the North Island of New Zealand. PhD thesis, Canterbury University. https://researcharchive.lincoln.ac.nz/items/4a7e2c92-5339-4d41-8036-eee155c9644d

Kain WM, Atkinson DS 1977. Development of resistant pasture and methods of pasture management for grass grub (*Costelytra zealandica* (White)) control, New Zealand Journal of Agricultural Research 20(4): 507-517. https://doi.org/10.1080/00288233.1977.10427367

Manaaki Whenua - Landcare Research 2019. S-map - New Zealand's national digital soil map. 10.7931/L1WC7. https://datastore.landcareresearch.co.nz/dataset/s-map

McNeill MR, Tu X, Ferguson CM, Ban L, Hardwick S, Zhang R, Barratt BIP, Zhang Z 2021. Diversity and impacts of key grassland and forage arthropod pests in China and New Zealand: A review of IPM and biosecurity initiatives. *Neobiota* 65: 137-168. https://doi.org/10.3897/neobiota.65.61991

Pottinger RP 1968. Comments on the ecology of grass grub and porina caterpillar. Proceedings of the New Zealand Grassland Association 30: 102-113. https://doi.org/10.33584/jnzg.1968.30.1247

Radcliffe JE 1970. Some effects of grass grub (*Costelytra zealandica* (White)) larvae on pasture plants, New Zealand Journal of Agricultural Research 13(1): 87-104. https://doi.org/10.1080/00288233.1970.10421199

Simpson W, Schmid J, Singh J, Faville M, Johnson R 2012. A morphological change in the fungal symbiont *Neotyphodium lolii* induces dwarfing in its host plant *Lolium perenne*. Fungal Biology 116: 234-240. https://doi.org/10.1016/j.funbio.2011.11.006

Stewart KM 1986. Control of grass grub (*Costelytra zealandica*) by cultivation in spring or summer. New Zealand Journal of Experimental Agriculture 14(1): 83-87. https://doi.org/10.1080/03015521.1986.10426129

Stewart KM, Stockdill SMJ 1972. Two year life cycle in grass grub and its implications on control. Proceedings of the 25th New Zealand Weed and Pest Control Conference: 244-247. https://doi.org/10.30843/nzpp.1972.25.8977

Van Toor RF, Dodds KG 1994. Assessment of grass grub (*Costelytra zealandica* (White)) (Coleoptera: Scarabaeidae) damage in ryegrass-white clover pasture in Southland. New Zealand Journal of Agricultural Research 37(1): 99-105. https://doi.org/10.1080/00288233.1994.9513046